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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/254,563	03/05/99	BRONSHTEIN V	UPTINC.015A

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EXAMINER	
SAUCIER,S	
ART UNIT	**PAPER NUMBER**
1651	8

DATE MAILED: 01/04/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/254,563	Bronshtein
Examiner	**Group Art Unit**
Sandra Saucier	1651

[X] Responsive to communication(s) filed on *Oct 3, 2000*

[X] This action is **FINAL.**

[ ] Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *1* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

[X] Claim(s) *1, 4-10, 12-17, 25, and 26* is/are pending in the applicat

Of the above, claim(s) _____ is/are withdrawn from consideration

[ ] Claim(s) _____ is/are allowed.

[X] Claim(s) *1, 4-10, 12-17, 25, and 26* is/are rejected.

[ ] Claim(s) _____ is/are objected to.

[ ] Claims _____ are subject to restriction or election requirement.

Application Papers

[ ] See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

[ ] The drawing(s) filed on _____ is/are objected to by the Examiner.

[ ] The proposed drawing correction, filed on _____ is [ ] approved [ ] disapproved.

[ ] The specification is objected to by the Examiner.

[ ] The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

[X] Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

[X] All [ ] Some* [ ] None of the CERTIFIED copies of the priority documents have been

[ ] received.

[ ] received in Application No. (Series Code/Serial Number) _____ .

[X] received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

[ ] Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

[X] Notice of References Cited, PTO-892

[ ] Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

[ ] Interview Summary, PTO-413

[ ] Notice of Draftsperson's Patent Drawing Review, PTO-948

[ ] Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

DETAILED ACTION

Claims 1, 4-10, 12-17, 25 and 26 are pending and are considered on the merits.

Information Disclosure Statement

The listing of the references on PTO 1449 is incomplete. A proper citation includes author, title, journal, volume, number, inclusive pages, (month), YEAR. The citation is missing the YEAR OF PUBLICATION of the article.

MPEP37 CFR 1.98(b) requires that each U.S. patent listed in an information disclosure statement be identified by patentee, patent number, and issue date. Each foreign patent or published foreign patent application must be identified by the country or patent office which issued the patent or published the application, an appropriate document number, and the publication date indicated on the patent or published application. Each publication must be identified by author (if any), title, relevant pages of the publication, date and place of publication. The date of publication supplied must include at least the month and year of publication, except that the year of publication (without the month) will be accepted if the applicant points out in the information disclosure statement that the year of publication is sufficiently earlier than the effective U.S. filing date and any foreign priority date so that the particular month of publication is not in issue. The place of publication refers to the name of the journal, magazine, or other publication in which the information being submitted was published.

Citations 1, 4, 5 and 7 on the IDS filed 6/7/99 are incomplete and have not been initialed.

Specification

This application does not contain an abstract of the disclosure as required by 37 CFR 1.72(b). An abstract on a separate sheet is required.

No abstract ON A SEPARATE SHEET has been submitted.

Claim Rejections - 35 USC § 112

INDEFINITE

Claim 1, 4-10, 12-17, 25 and 26 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites "vitrifying the dehydrated specimen, without freezing, by cooling to a refrigeration or higher storage temperature.".

It is uncertain just what temperature constitutes "a refrigeration temperature". It cannot be determined what exact temperature is the lower limit of the phrase "a refrigeration temperature", because refrigeration is defined by Gould's Medical Dictionary to be "The act of lowering the temperature of a body by conducting away its heat to a surrounding cooler substance"; therefore, a refrigeration temperature is interpreted to be any temperature where heat is conducted away from a body. The upper limit of this phrase "or higher" cannot be clearly understood, as it may mean that no lowering of the temperature of the initial temperature of the specimen takes place. It is not possible that vitrification takes place without cooling or lowering of the initial temperature of the specimen. Thus, this phrase is not interpretable.

Claim 1 is unclear because it appears that a cooling or lowering of the temperature occurs, but no mention of the starting temperature is present in the claim; therefore, one cannot determine what cooling means because there is no starting temperature or step from which to measure. "Cooling" is a relative term with no reference point.

Claim 13 remains indefinite because their is no precedent for the recitation of "permeating rehydration cryoprotectant".

Claims 25 and 26 are indefinite because they state that the non-permeating co-solute can be a polysaccharide. However, in claim 5 polysaccharides such as Ficoll, dextrans starches are said to be non-permeating cryoprotectants. These two groups of compounds, non-permeating co-solute and non-permeating polymeric cryoprotectant appear to be overlapping. Thus, the combination of only two compounds, a polysaccharide and a permeating co-solute such as DMSO or glycerol would appear to fulfill the claim limitations for all three groups. Please clarify.

NEW MATTER

Claims 1, 4-10, 12-17, 25 and 26 are rejected under 35 U.S.C. 112, first

paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

Insertion of "equilibrating" in claims 9 and 10 appears to be new matter. Equilibration is the point where the concentrations of permeating compounds are equal on the inside and the outside. The specification does not say that this equilibrium point is reached, it merely appear to say that "the time of cell equilibration is significantly increased". This appears to mean that it takes longer to reach the equilibrium point when the inventive mixture is used than other mixtures. The method as disclosed in the specification does not state that the cell reaches an equilibrium point which is indicated by the recitation in the claim of "equilibrating" the specimen. Thus, the meaning of the recitation about "equilibration time" in the specification is not the same as in the method as presently claimed. Please cancel "equilibrating".

Insertion of the phrase "without freezing" does not appear to have support in the as-filed specification. It is noted that applicant has not pointed to the passage where this phrase occurs. Please cancel "without freezing".

Insertion of "about" in claim 7 is new matter because it broadens the range. Please point to the place in the specification where "between about 0.1and 0.7 mol/l" is found or please cancel "about".

This is a matter of written description, not a question of what one of skill in the art would or would not have known. Applicant must limit claims to the material within the four corners of the as-filed specification. If the material is not present, the material is new matter.

Claim Rejections - 35 USC § 102

Claims 1, 4-7, 16, 25 remain/are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Titterington *et al.* [U].

The claims are directed to a method of preserving cells or tissue by equilibrating and dehydrating a cell or tissue with a solution comprising a

1) non-permeating co-solute (amino acids or derivatives, betaine,

carbohydrate such as {aldose monosaccharide, ketose monosaccharide, amino sugar, alditol, inositol, aidonic, uronic or aldaric acid}, a sugar alcohol, disaccharide or polysaccharide),

2) a permeating cryoprotectant (DMSO, ethylene glycol, propylene glycol or glycerol) and

3) a non-permeating polymeric cryoprotectant (dextran, starch, PEG, PVP, Ficoll, peptides),

vitrifying the specimen by cooling to a refrigeration or higher storage temperature.

Titterington *et al.* disclose a method of cryopreservation of mouse embryos comprising treating the embryos with a composition containing 1) sucrose (0.75M), 2) 50% glycerol and 3) 50% Percoll and put in liquid nitrogen to vitrify the embryos.

Since refrigeration is defined by Gould's Medical Dictionary to be "The act of lowering the temperature of a body by conducting away its heat to a surrounding cooler substance" and also by Grant and Hack's Chemical Dictionary to be "The production of cold; lowering of the temperature of a body by conducting away its heat.", the act of placing embryos in liquid nitrogen, which is a surrounding cooler substance, is considered to fulfill the claim limitations, contrary to applicant's arguments.

Claims 1-6, 9, 10, 16, 18-24, 25 remain/are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Rall *et al.* [V].

Rall *et al.* disclose a method for cryopreserving embryos comprising treating the embryos with a composition comprising 1) glucose, 2) glycerol 6.5M, 3) BSA. The concentration of the components of the solution is increased stepwise (page 682). The embryos are vitrified by cooling to -196C.

See discussion above regarding "refrigeration temperature".

Claims 1, 4-7, 9, 12-16, 25 and 26 remain/are rejected under 35 U.S.C. 102(b) as being clearly anticipated by US 5364756 [D].

US 5364756 disclose a method of preserving cells (col. 4, l. 60) by contacting the cells with a solution comprising 1)raffinose, 2) DMSO and 3) dextran (col. 13, tables). The sample is dried using the temperature program in col. 17, l. 32-46. The sample is stored and rehydrated with a solution containing 1) DMSO, 2) trehalose, 3) dextran, col. 20, table. The rehydration solution is then diluted with culture medium.

In example 4, the red cell sample is contacted with 1% glycerol, suspended in 1% dextran, treated with a vitrification solution, VS1, 0.025M DMSO, 0.025M propylene glycol,0.0125 butanediol, 0.5% raffinose, 0.3% trehalose, 0.3% sucrose, 0.6% PVP, 0.6% dextran, nebulized, and the temperature of the nebulized sample brought down to -190C. The samples were then dried by molecular distillation and stored under vacuum. In col. 14 is described that the most preferred procedure for cooling is a vitrification procedure. Thus, it is reasonable to assume that in the examples, the most preferred procedure is exemplified.

Applicant argues that '756 discloses freezing, not vitrification of the specimen during processing.

If in example 4, the concentration of the vitrification materials is not great enough to permit vitrification at about -160C to -190C, applicant has not so argued or demonstrated. Also, if the concentration of all of the non-permeating co-solutes does not fall within applicant's claimed range of 0.1-0.7M, applicant has not so argued or demonstrated. Steps 1-3 of example 4 appear to be the same/similar as applicant's instant claimed physical steps. Whether or not the inventor of '756 knew the correct state of the sample, that is if the sample was in a frozen or vitrified state does not detract from the description of the actual physical steps. Belief or erroneous theoretical understanding does not detract from methods in a patent which have actual, physical manipulations described. Patents are not peer-review publications, where correct theoretical understanding of the process is of great importance. What is essential in a patent disclosure or claim is the physical performance of the steps which results in a product. Even though some of the characteristics of the product might not be fully appreciated or disclosed by the patentee, this does not detract from the teaching of method steps in the patent when a patent is used as a reference.

If in fact, the concentration of cryoprotectant and preparation of the cells described in '756 does not lead to a vitrified state at step 3, this rejection might be overcome. If the concentration of co-solutes does not fall within applicant's concentration parameters, insertion of those parameters into the independent claim may overcome this rejection. Also, please note that in column 14, the cooling parameters of the suspension in the most preferred embodiment lead to vitrification. Further, please note that applicant's claims are open to further steps such as drying or nebulization prior to vitrification or to the further addition of other cryoprotectants such as glycerol in step 1b of example 4. Applicant's arguments that the nebulized sample of example 4 is not in a vitrified state, rather in a frozen state @ -190C or -160C are unpersuasive.

Claims 1, 4-7, 9, 10, 12-17, 25 and 26 remain/are rejected under 35 U.S.C. 102(e) as being clearly anticipated by US 5800978 [E].

US 5800978 discloses a cryopreservation medium comprising 0.5M glycerol, 7.5% BSA and 0.3M glucose or 5% glucose, 10% FCS, 20% Dextran40 (Table 1). A method of cryopreservation or red cells using 5% glucose (permeant), 10% sucrose (impermeant) and 20% PVP (impermeant) (buffer #8 Table 2) and other three component combinations of cryoprotectants is shown. The general principle of using a three component cryoprotectant buffer comprising a permeant (monosaccharides or polyalcohols), impermeant (disaccharide) and a high molecular weight polymer is disclosed in Example 1. Example 1 also states that the Tg of the solution MUST BE ADJUSTED to that the Tg exceeds -45C and preferably is above -25C for convenient frozen storage.

In example 4, 5.7 mls of cryoprotectant buffer was added to 5 mls of cells in dextrose-saline then an additional 5.7 mls of buffer was added prior to lowering the temperature to -80C. This is a one step increase in concentration of the buffer prior to lowering the temperature. During thawing, the cells were reconstituted by dilution of the freezing buffer with a reconstitution buffer comprising PVP and glucose (example 4). In table 11, red cells after dehydration are stored at 80°C for 4-6 days. In Table 10, a mixture of glycerol, glucose, lactose and HES is added to red cells prior to lyophilization.

Contrary to applicant's arguments, this reference discloses adding a cryopreservation buffer with a Tg of -30C to red cells with mixing in two steps. Applicant argues that no disclosure of "dehydrating" is seen. However, the

"dehydrating" occurs as an inherent result of mixing the cryoprotectant buffer which has applicant's three classes of compounds. Thus, applicant's argument is not persuasive. Applicant argues that the reference discloses "freezing" not vitrification. However, the reference discloses quickly lowering the temperature of the sample to -80C which is below the Tg of the cryoprotectant about -30C. Therefore, the sample is vitrified. Whether of not the reference appreciates that the sample is not "frozen" or has used improper language to describe a state which results from the performance of the disclosed steps is of little import. Lowering the temperature of a vitrifiable mixture below its vitrification point results in a vitrified sample whether or not the true state of the sample is appreciated or conveyed in the text.

Claim Rejections - 35 USC § 103

Claims 1, 4-10, 12-16, 25 and 26 are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over US 5364756 [D] in view of US 5217860 [C] taken with US 4865871 [E] or Rall *et al.* [V] and US 5879876 [F].

The claims are directed to a method of preserving cells or tissue by equilibrating and dehydrating the cell or tissue with a solution comprising a 1) non-permeating co-solute (amino acids or derivatives, betaine, carbohydrate such as {aldose monosaccharide, ketose monosaccharide, amino sugar, alditol, inositol, aidonic, uronic or aldaric acid}, a sugar alcohol, disaccharide or polysaccharide), 2) a permeating cryoprotectant (DMSO, ethylene glycol, propylene glycol or glycerol) and 3) a non-permeating cryoprotectant (dextran, starch, PEG, PVP, Ficoll, peptides), vitrifying the sample, without freezing by cooling to a refrigeration temperature of higher. Dependent claims require an increase in the concentration of the cryopreservative solution or a decrease in the concentration of the rehydration solution.

The primary reference of US 5364756 lacks the use of increasing concentrations of cryoprotectant prior to freezing.

US 5217860 disclose a method of cryopreserving tissue comprising adding a solution containing 1) formamide, 2) DMSO and increasing the concentration of the solution according to a desired profile.

US 4865871 discloses the details of the protocol used in US 5364756 (col. 11, l. 1-18).

US 5879876 discloses a general method of diluting a cryoprotectant from a thawed tissue using Plasmalyte and mannitol (ex. 2). Plasmalyte contains glucose which is a permeant.

The other references are relied upon as explained above.

The use of an increasing concentration of cryopreservatives prior to cooling the temperature of a sample in the place of the one step addition method as disclosed by US 5364756 would have been obvious when taken with US 5217860 or Rall *et al.* which disclose stepwise increases in the concentration of cryoprotectants prior to cooling in order to decrease osmotic stress in cells/tissues. The stability of the processed tissue as disclose by US 5364756 is assume to be the same as the claimed stability because the process which produces the stable product, as claimed, is essentially the same as the process disclosed in the prior art. In particular, see US 4865871 which discloses a method of sublimation and storage of biological samples after the addition of cryoprotectants. US 5364756 also discloses diluting the cryoprotectant solution after reconstitution (col. 20, table). US 5879876 discloses that gradual dilution of the cryoprotectant after thawing lessens osmotic shock of the tissue.

The method of increasing concentrations of cryoprotectants prior to freezing and decreasing concentrations of rehydration solutions during reconstitution or thawing is well known in the art. The cryoprotectant compositions as well as the rehydration compositions are also well known in the art. Neither the claimed method nor the claimed compositions are allowed.

One of skill in the art would have been motivated at the time of invention to make this substitution in order to obtain the results as suggested by the references with a reasonable expectation of success. The claimed subject matter fails to patentably distinguish over the state of the art as represented by the cited references. Therefore, the claims are properly rejected under 35 U.S.C. § 103.

Applicant argues that all of the cited references teach minimizing damage caused by ice crystallization, none of the references teach or even suggest the use of such formulations for vitrification of dehdrated specimens by cooling at a refrigeration or higher storage temperature. Please see the examiner's comments above with regard to the anticipatory rejection over '756.

Conclusion

Applicant's amendment necessitated the new grounds of rejection presented in this Office action. Accordingly, THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1651. The supervisor for 1651 is M. Wityshyn, (703) 308-4743.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sandra Saucier whose telephone number is (703) 308-1084. Status inquiries must be directed to the Service Desk at (703) 308-0196. The number of the Fax Center for the faxing of papers is (703) 308-4227.

Sandra Saucier
Primary Examiner
Art Unit 1651
December 22, 2000